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San Francisco, CA 94107-0946

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14. ABSTRACT

The main goal is to develop a PET imaging agent based on modifying the peptidomimetic PSMA inhibitor which will result in improved tumor uptake and clearance mechanism. Different fluorination approaches were attempted with PSMA module compounds such as direct labeling, cupper free chemistry and the use of prosthetic group (SFB). The direct labeling approach has been eliminated as an option due to the instability of the fluorination. As for the cupper free chemistry labeling approach, the final purification step is in progress to provide a final product with higher specific activity (less mass for better imaging). In addition, the production of SFB has been attempted for labeling the module compounds as well and the work is ongoing. Once the labeling approaches are established, and then the labeling of the modified PSMA inhibitor analogues will be investigated in vitro as well as in vivo.

15. SUBJECT TERMS
PSMA, Fluorine-18, Click Chemistry, SFB, PET, Prostate Cancer, CW22Rv1, PC3

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INTRODUCTION:

This training grant focuses on modification and the development of novel diagnostic agents for prostate cancer that will take advantage of the efficiency and specific affinity of small-molecule inhibitors of Prostate Specific Membrane Antigen (PSMA). The main hypothesis is to develop a PET imaging agent based on modifying the peptidomimetic PSMA inhibitor which will result in improved tumor uptake and clearance mechanism.

BODY:

The PI had accomplished most, if not all, of the training outlined in the approved Statement of Work. Starting with being trained on radiolabeling small molecules using fluorine-18 gas at the University of California Davis; however this radiolabeling approach of the PSMA analogues was halted as our in-house fluorine-18 gas production system is currently not available for use. Also, the PI had observed the process of the cyclotron facility operation and production of short-lived isotopes and was trained on the production of radiopharmaceuticals for human injection with a licensed radiopharmacist at the cyclotron facility. During the award period, three undergraduates from the University of California Berkeley and one graduate student from UCSF were trained and mentored by the PI in the lab.

In addition, the PI had attended multiple meetings that allowed her to expand her knowledge in cancer imaging and in particular prostate cancer imaging. The PI attended the Prostate Cancer Symposium that was held at the Mid-Winter SNM meeting in January of 2011. She had also attended the Cancer Research Imaging Camp by NCI that was held in June 2011 at the Washington University of St. Louis. During the Imaging Camp, the PI was introduced to different imaging modalities by the expert on their fields. In April 2012, the PI had attended the Prostate Cancer Retreat at UCSF which she had learned about the latest in the Prostate Cancer Research being held. She attended and presented her work at the 2013 UCSF Prostate Cancer Retreat. Meanwhile, the PI has been attending regular meetings with her collaborators at Washington State University for updates twice a month, and weekly meetings with her mentors at University of California San Francisco.

As for the research accomplishments, Aim #1 was accomplished. The main goal of this aim is to find a high yielding and efficient fluorination labeling approach of chloropyrimidine module compounds (Figure 1). The

PI was able to achieve direct nucleophilic fluorine-18 labeling of chloropyrimidine module compounds that were done at room temperature compared to the required harsh labeling conditions of fluorine-18, however, the fluorination was not stable at physiological conditions. Different substitution groups on the module compounds were investigated in an attempt to stabilize the labeling but that was not successful, as a result the next step was to try a different labeling approach.

The main goals of aims 2 and 3 were accomplished. For aim 2 work, we have tried radiolabeling our PSMA inhibitor analogues with ^{68}Ga but it was unsuccessful as the ^{68}Ga was not chelating to DTPA or DOTA. Instead, ^{68}Ga was chelating to the five carboxylic acid functional groups present on the analogues diminishing the binding affinity to PSMA. We have tried protecting the carboxylic acid functional groups on the lead analogues but that have added an additional deprotection step after the labeling step which lowered the radiochemical yield and specific activity. Therefore, the best radiolabeling route to fluorinate our PSMA inhibitor analogues was with the use of the prosthetic group [^{18}F]N-succinimidyl-4-fluorobenzoate ([^{18}F]SFB). Using this approach, the prosthetic group is radiolabeled with F-18 then it will be coupled/conjugated to our biomolecule. Three of our PSMA inhibitor analogues (TG-97, AH-TG97 and AH2-TG97) that posed the highest binding affinity (Figure 2, left side) were used throughout this study.

The prosthetic group ([^{18}F]SFB) was synthesized as outlined in Scheme 1 with 10-15% radiochemical yield and >98% radiochemical purity. Now we were able to obtain [^{18}F]SFB using a Nebtis unit equipped with commercially available cassettes. The lead analogues were conjugated to [^{18}F]SFB under basic conditions at 40 °C for 10-15 min, yielding [^{18}F]FB-TG97, [^{18}F]FB-AH-TG97 and [^{18}F]FB-AH2-TG97 (Scheme 2), with 50-60% radiochemical yield and >98% radiochemical purity after HPLC purification. Product confirmation was done via an HPLC co-injection of the radiolabeled product along with a previously synthesized and characterized non-radioactive standard (Figure 2, right side) by our collaborators. All of the three radiolabeled analogues co-eluted with their standards confirming that the desired product was formed (Figure 3).

For aim 3, in order to determine the lead analogue for MicroPET/CT imaging studies, small scale biodistribution study were performed on all three lead analogues. We faced a problem with the LNCaP (PSMA positive) cell line so we used CW22RV1 (PSMA positive) cell line. CW22RV1 has 5-10 fold less of PSMA expression than LNCaP. If we saw tumor uptake with CW22RV1 tumor bearing mice then we would expect to have higher tumor uptake in LNCaP tumors. Biodistribution studies consisted of injecting mice bearing the CW22RV1 tumors with 50 uCi in 150 uL doses and removing the tumors, kidneys and a sample of the blood at 1 and 2 hours post injection of four mice at each time point. To determine PSMA specificity of the three lead analogues, 4 mice per analogue were pre-injected with 250 ug of a blocking dose (LW54, known to have high

affinity to PSMA) 60 min prior to administration of the tracer and the tumors, kidneys and a sample of the blood were removed at 2 hours post tracer injection.

Based on the biodistribution data, all three analogues ([^{18}F]FB-TG97, [^{18}F]FB-AH-TG97 and [^{18}F]FB-AH2-TG97) had high tumor uptake (Figure 4). The high kidneys uptake is also important as rodent kidneys have high PSMA expression which was observed with all three analogues (Figure 5). As for the blocking study, the tumor uptake was significantly blocked at around 56-67% (Figure 4) and the kidney's uptake was more significantly blocked at around 88-93% (Figure 5). The high tumor and kidneys uptake and the ability to significantly blocking the receptor with pre-injection of mass indicates that our lead analogues have high affinity and specificity to PSMA. The tumor : blood was investigated as early as 1 hour post injection of all three lead analogues (Figure 6) and the high ratio refers to the fast blood clearance which is desired for sparing the normal/non target organs from unnecessary radiation and enhances the PET images. We have done a second round of full biodistribution studies of all three analogues for a more accurate comparison of mice bearing CW22Rv1 tumors along with mice bearing PC3 tumor cell line for a negative control. The data is presented in Table 1 that confirms was previously seen with the first round of biodistribution studies. Our analogues exhibited high specificity and affinity to PSMA as there was little to no uptake observed in the non-target organs and PC3 tumors which does not express PSMA.

Imaging studies of all three lead analogues were performed on CW22RV1 (PSMA+) and PC3 (PSMA-) tumor bearing mice (Figure 6). Mice were injected with 200 uCi in 200 uL doses and MicroPET/CT images were taken at 2 h post injection. Tumor and kidney uptake of all three analogues was observed, and the kidney uptake is primarily due to the fact that rodent kidneys have high PSMA expression. Minimal to no uptake in other non-target organs and non-PSMA expression PC3 tumors was observed.

In addition to all the [^{18}F]SFB work done in aim 2, we have been working on optimizing the cupper free click chemistry prosthetic group. The goal of this work was to prepare [^{18}F]fluoroazides, prosthetic groups for click chemistry, from polymer-supported sulfonyl esters. We are working on optimizing the fluorination of the azido-alcohol precursor so we synthesized three different precursors with a UV active chromophore (Scheme 3) for characterization against a non-radioactive standard. The three azido alcohol precursors were loaded on insoluble polystyrene resin under inert conditions in 1:1 dichloromethane : pyridine as indicated in Scheme 4. Different fluorination ($^{18}F^-$) labeling conditions were investigated (Table 2). The optimal labeling conditions requires at least 10 mg of the precursor on the resin soaked for 5-10 min in 200 uL anhydrous DMF followed by the addition of $^{18}F^-$ solution in 100 uL anhydrous DMF containing 2 mg K_2CO_3 and 12.6 mg kryptofix heated at 150 C for 30 min either in the microwave or oil bath. Precursor 1 (Scheme 3) did not label. However,

precursor 2 did label/fluorinate but there was a non-radioactive mass peak observed on the HPLC trace indicating the presence of impurity that might have resulted from an elimination reaction as can be seen from the HPLC trace of the crude labeling reaction and the non-radioactive standard in Figure 7. This elimination product was not observed with precursor 3 that was fluorinated under the optimized conditions.

KEY TRAINING ACCOMPLISHEMNTS:

1. Mentored and trained total of three undergraduate students from the University of California Berkeley
2. Mentored and trained a graduate (master) student from UCSF
3. Attending conference calls via Skype with collaborators twice a week
4. Attending weekly group meetings with mentor
5. Trained on the production and labeling of fluorine-18 gas
6. Observed the process of operating and producing short lived isotopes at the cyclotron
7. Trained on the production of radiopharmaceuticals for human injection
8. Attended the Prostate Cancer Symposium at the Mid-Winter SNM meeting (Jan 2011)
9. Attended the Cancer Research Imaging Camp by NCI at Washington University St. Louis (June 2011)
10. Attended the Prostate Cancer Retreat at UCSF (April 2012 and September 2013)

KEY RESEARCH ACCOMPLISHEMNTS:

1. Performed direct fluorine-18 labeling of module compounds
    a. Not stable under physiological conditions
2. Optimized the radiosynthesis of the prosthetic group fluorine-18 succinimidyl-4-fluorobenzoate ([^{18}F]SFB)
    a. Synthesizing the SFB prosthetic group for labeling using a different synthesis route than the usual method
3. Optimized the conjugation conditions of [^{18}F]SFB to our PSMA inhibitor analogues
    a. Consistent reproducible results were obtained of [^{18}F]SFB conjugation
    b. Final radiolabeled product was confirmed for all analogues via RP-HPLC con-injection with the characterized nonradioactive standards (both nonradioactive and radioactive products co-eluted)
    c. High radiochemical yields were obtained (50-60%) decay corrected
    d. High radiochemical purity (>98%) as confirmed by RP-HPLC
4. Conducted two full rounds of in vivo studies
    a. Biodistribution of PSMA positive tumor bearing mice were conducted and blocking studies to confirm specificity to our targeting receptor (PSMA) of the three radiolabeled lead analogues

b. Imaging studies of PSMA positive and negative tumor bearing mice were conducted of the three radiolabeled leading analogues

5. Investigated cupper free click fluorine-18 labeling

a. Fluorinating the compound of interest off the resin which allowed for simplified purification step

b. Using the azido resin to clean up the final product of unreacted compounds to obtain higher specific activity product

6. Investigated additional azido alcohol precursor on resin for cupper free click chemistry

a. Loading three different azido alcohol precursors with UV chromophore on resin

b. Fluorinating the loaded azido alcohol precursors off the resin which allowed for simplified purification step

c. Characterization of the radiolabeled reaction via co-injection of the characterized non-radioactive standard and crude F-18 reaction to determine the radiolabeling yields, confirmation of product formation and possible side products.

i. Precursor 1 did not fluorinate

ii. Precursor 2 fluorinated with yields ~40% but there was a major elimination product (impurity/by product)

iii. Precursor 3 fluorinated with yields ~30-40%

7. Trained on operating the Ga-68 generator

a. Performing Ga-68 labeling of model compounds

REPORTABLE OUTCOMES:

1. "Potential One Step Labeling $^{18}F^-$ Prosthetic Group"

   Abstract for an oral presentation at the 243rd ACS National Meeting on March 25th 2012 *(Glenn T. Seaborg Award for Nuclear Chemistry: Symposium in Honor of Silvia S. Jurisson)*

2. "Radiofluorination of polymer-supported sulfonyl esters: Efficient preparation of labelled compounds"
   Abstract for a poster presentation at the 20th ISRS Meeting on May 11th-18th 2013 (Jeju, South Korea)

3. "A high affinity ^{18}F-labeled phosphoramidate peptidomimetic inhibitor as a PSMA-targeted PET imaging agent for prostate cancer"
   Abstract for a poster presentation at the 20th ISRS Meeting on May 11th-18th 2013 (Jeju, South Korea)

4. "Fluorine-18 labeled PSMA-targeted phosphoramidate inhibitors as potential PET imaging agents for prostate cancer"

   Abstract for an oral presentation at the 60th SNM Meeting on June 8th-12th 2013 (Vancouver, BC)

5. "Fluorine-18 labeled PSMA-targeted phosphoramidate inhibitors as potential PET imaging agents for prostate cancer"

   Abstract for a poster presentation at UCSF Prostate Cancer Research Retreat on September 9th 2013

CONCLUSION:

In conclusion, we were able to synthesize three modified lead PSMA inhibitor analogues with high binding affinity to PSMA. The [^{18}F]SFB was optimized and its conjugation to the lead analogues was performed in high radiochemical yields and purity. Based on the in vivo data, AH-TG97 and AH2-TG97 expressed favorable characteristics as non-invasive prostate cancer PET imaging agents due to their high tumor uptake, retention, clearance properties and tumor to blood ratio. Therefore, those two analogues are promising candidates for the clinical trial.

Also, as outline in aim 2, we are working on optimizing other methods to radiolabel our lead compounds. Cupper free click chemistry is a very attractive method and we have optimized the fluorination process of the azido precursor that will be clicked on our PSMA lead analogues. We have successfully synthesized [^{18}F]fluoro-azido prosthetic precursors from resins (polystyrene-bound sulfonyl esters). This method simplifies post labeling purificatition and provides a solution void of reactive precursor material. Optimal labeling conditions: DMF with 2 mg of K_2CO_3/6.4 mg of kyrptofix (K_{222}) and microwave heating at 150 ºC for 30 min.

The writing of two manuscripts is in progress.

REFERENCES:

None

APPENDICES:

SUPPORTING DATA

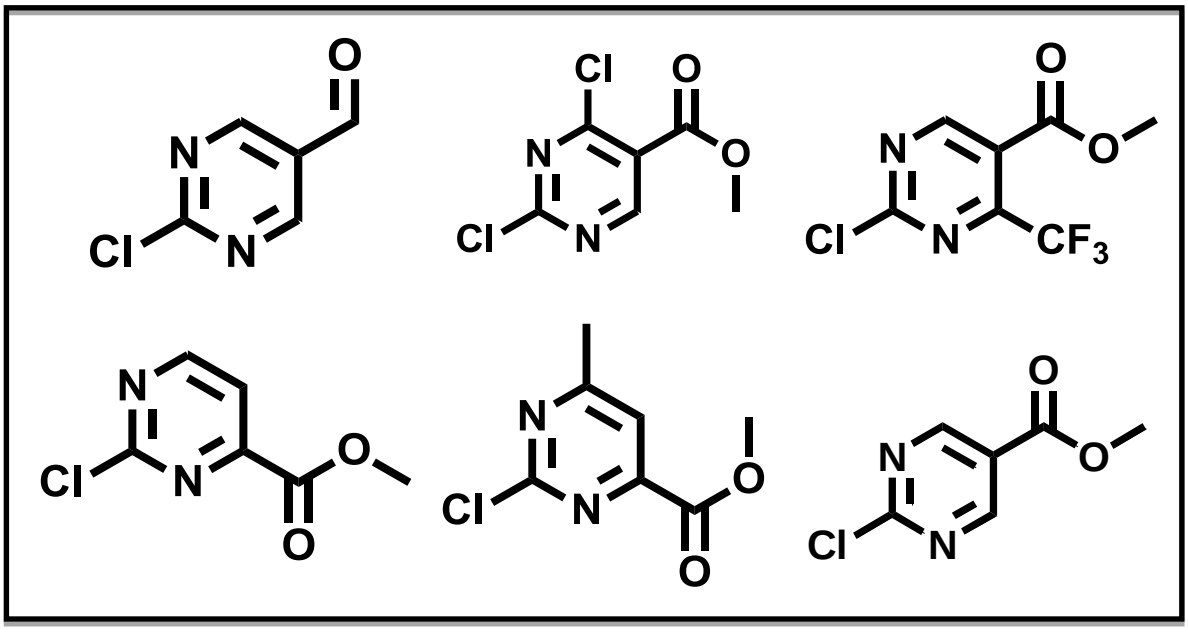


Figure 1. Chloropyrimidine compounds investigated for direct fluorine-18 nucleophilic labeling.

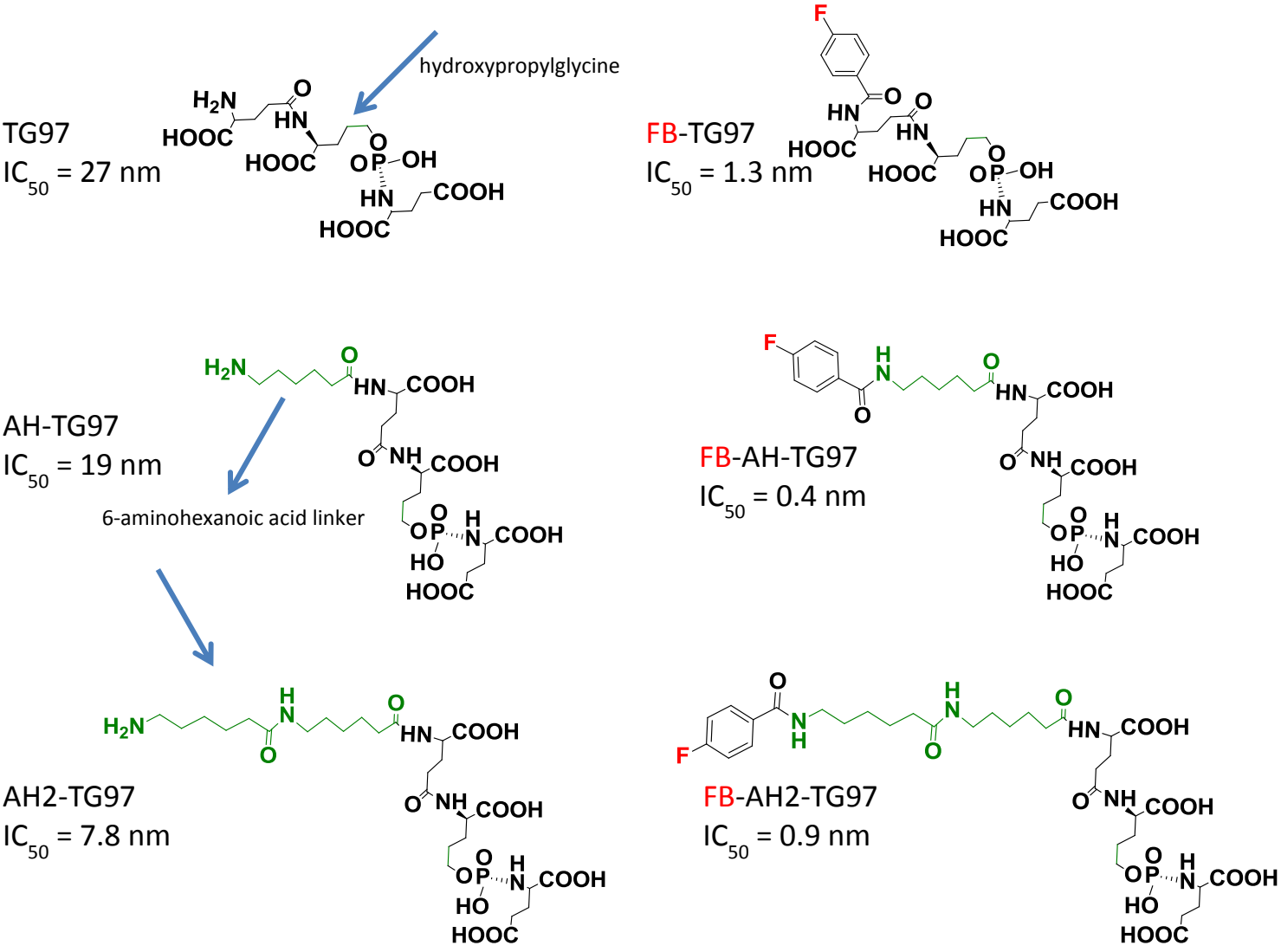
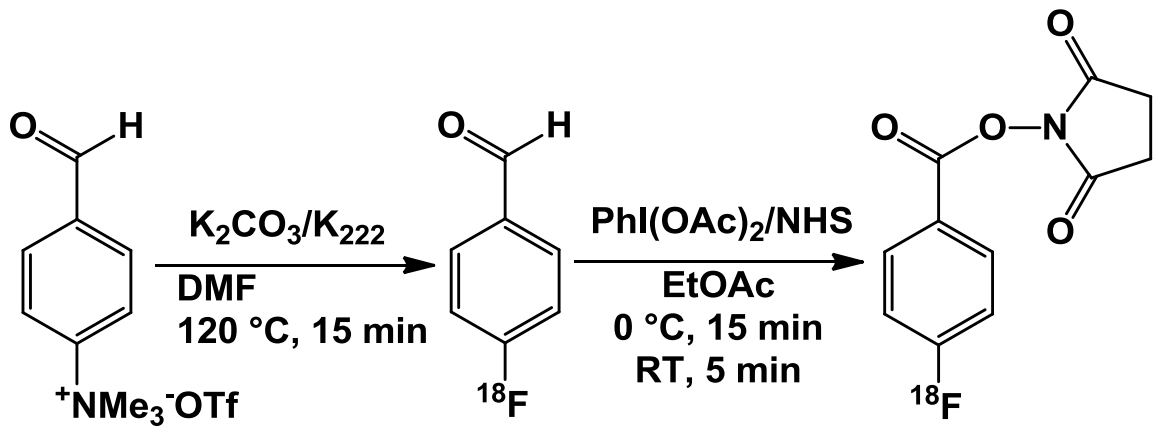
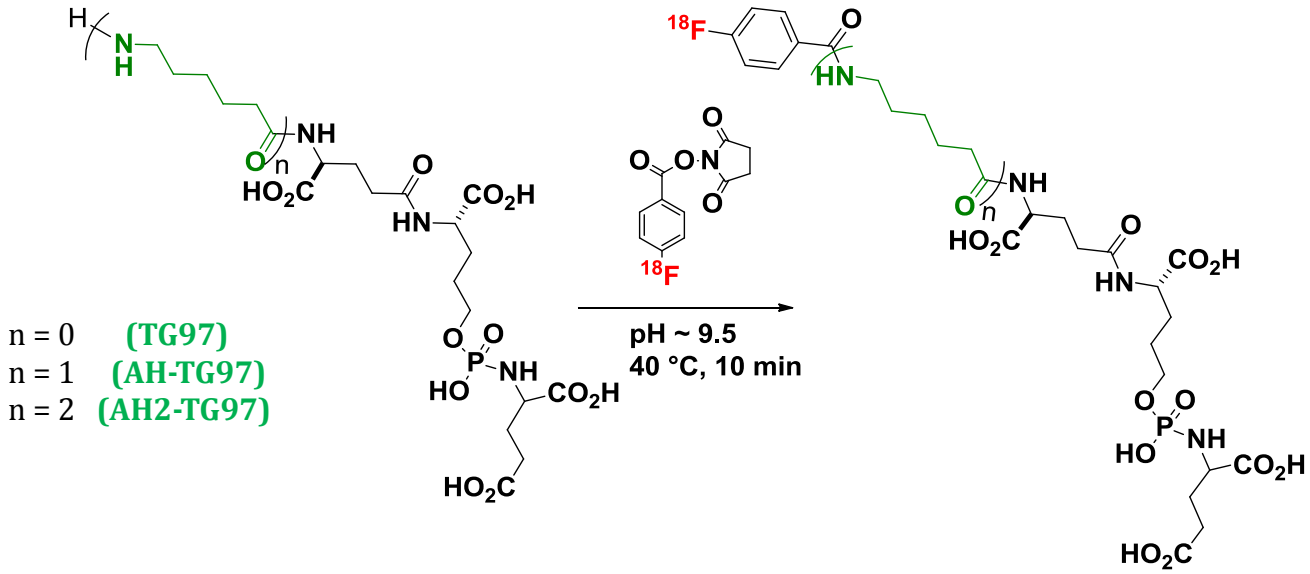


Figure 2. Three Leading PSMA Inhibitor Analogues: posing the highest binding affinity to PSMA. **Left side:** the analogues with amine available for the prosthetic group (SFB) conjugation/coupling with their IC_{50} (binding affinity) values. **Right side:** the conjugated analogues with their IC_{50} values.



Scheme 1. [^{18}F]N-succinimidyl-4-fluorobenzoate ([^{18}F]SFB)



Scheme 2. [^{18}F]SFB to the PSMA inhibitor lead analogues

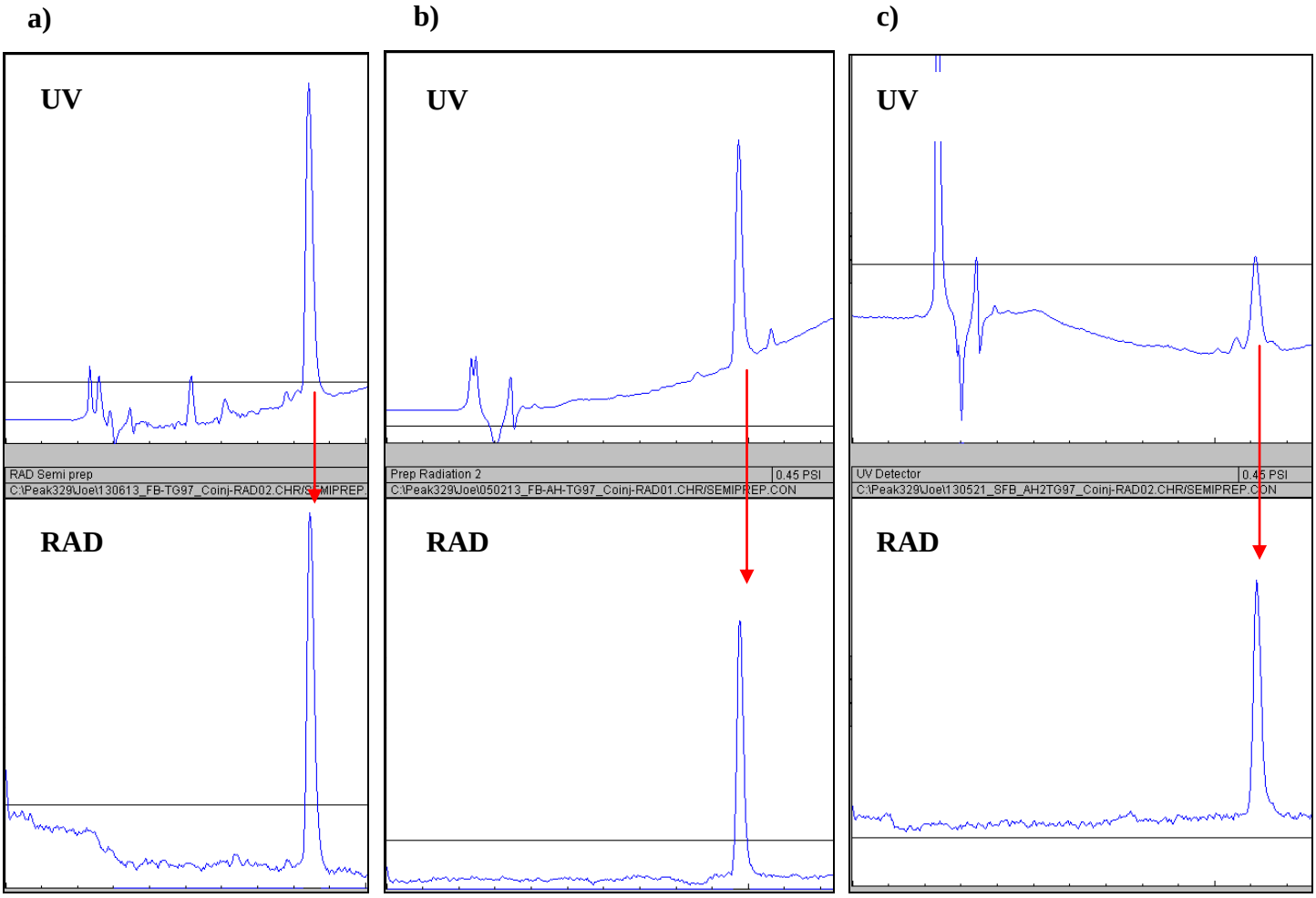


Figure 3. HPLC traces: co-injection of [^{18}F]SFB conjugated lead analogues with their complementary non-radioactive standards. Top traces are UV channel at 254 nm of the non-radioactive standard and the bottom traces are the RAD channel of the [^{18}F]FB-TG97 analogues **a)** [^{18}F]FB-TG97 **b)** [^{18}F]FB-AH-TG97 **c)** [^{18}F]FB-AH2-TG97

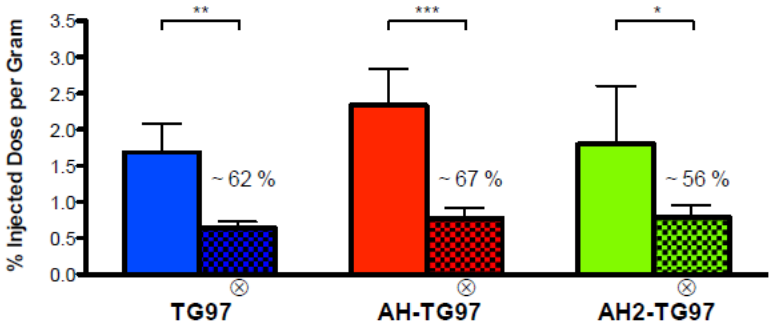


Figure 4. Tumor Uptake: data at 2 hours post injection. ⊗ Blocking studies: pre-injecting the mice with 250 ug of the first generation PSMA inhibitor lead compound with known high affinity to PSMA.

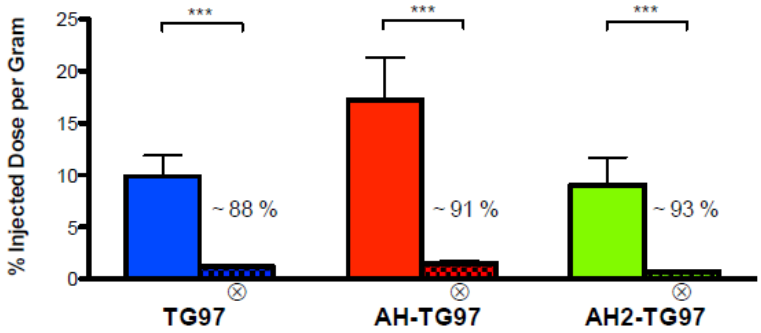


Figure 5. Kidney Uptake: data at 2 hours post injection. Rodent kidney have high PSMA expression. ⊗ Blocking studies: pre-injecting the mice with 250 ug of the first generation PSMA inhibitor lead compound with known high affinity to PSMA.

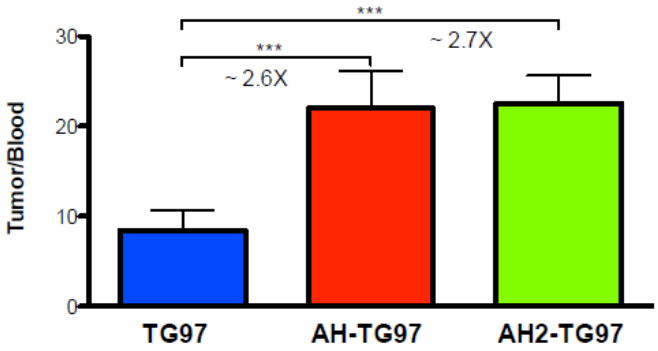


Figure 6. Tumor:Blood data at 1 hours post injection.

Tissue	[^{18}F]TG97			[^{18}F]AH-TG97			[^{18}F]AH2-TG97		
	CWR22Rv1		PC3	CWR22Rv1		PC3	CWR22Rv1		PC3
	1 h	2 h	2 h	1 h	2 h	2h	1 h	2 h	2h
Blood	0.15±0.07	0.07±0.02	0.08±0.04	0.17±0.05	0.04 ± 0.02	0.05±0.02	0.12 ± 0.03	0.03 ± 0.01	0.02±0.01
Heart	0.75±0.32	0.50±0.05	0.36±0.21	0.34±0.11	0.17±0.06	0.21±0.07	0.23 ± 0.05	0.06 ± 0.01	0.07±0.03
Lung	0.65±0.34	0.43±0.11	0.29±0.09	0.43±0.09	0.21±0.10	0.27±0.08	0.25 ± 0.07	0.11 ± 0.04	0.09±0.02
Liver	0.83±0.23	0.5 ±0.11	0.44±0.27	0.49±0.11	0.29±0.08	0.28±0.05	0.49 ± 0.07	0.25 ± 0.04	0.25±0.04
Kidneys	8.94±2.93	9.97±2.81	5.46±2.41	24.38±3.72	21.54±6.12	18.98±4.75	5.87 ± 0.67	7.13 ± 1.45	4.44±1.03
Spleen	1.18±0.08	0.87±0.16	0.76±0.35	1.02±0.04	0.84±0.30	1.38±1.05	0.32 ± 0.12	0.19 ± 0.03	0.14±0.03
Bone	0.46±0.04	0.5 ±0.42	0.24±0.06	0.38±0.09	0.23±0.15	0.17±0.04	0.45 ± 0.12	0.21 ± 0.11	0.14±0.04
Muscle	0.29±0.09	0.19±0.01	0.17±0.04	0.12±0.04	0.10±0.06	0.06±0.01	0.15 ± 0.04	0.08 ± 0.02	0.03±0.01
Tumor	1.54±0.40	1.57±0.45	0.40±0.17	3.16±0.39	1.65±0.32	0.38±0.03	2.92 ± 0.30	1.86 ± 0.14	0.27±0.07
Tumor : Blood	9.88±5.21	25.61±14.99	N/A	20.01±9.06	63.60±18.08	N/A	24.21±3.21	69.60±15.72	N/A

Table 1. Biodistribution of [^{18}F]TG97, [^{18}F]AH-TG97 and [^{18}F]AH2-TG97 as determined by radioactivity assays in PSMA+ CWR22Rv1 tumor-bearing mice (n = 4 in each group). Tissues were harvested at 1h and 2h post injection. Uptake values are expressed as %ID/g of tissue

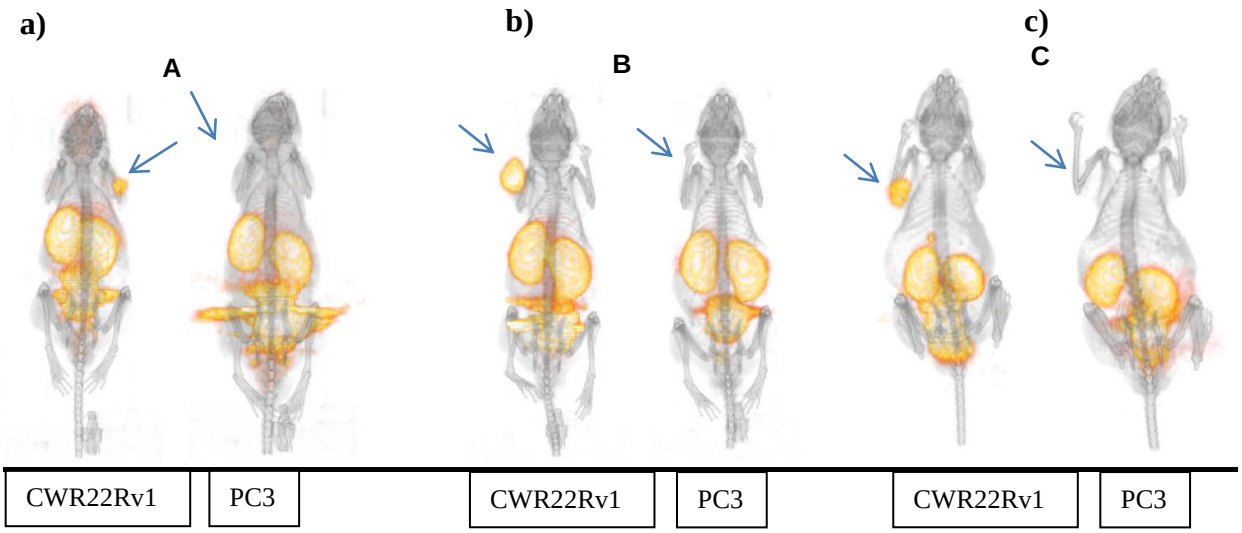
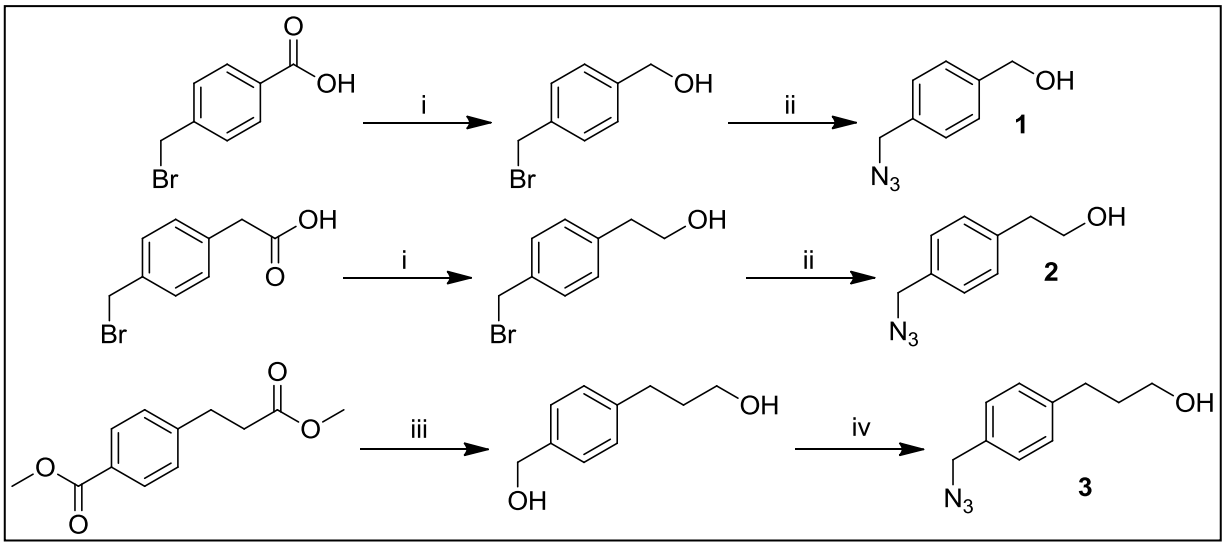
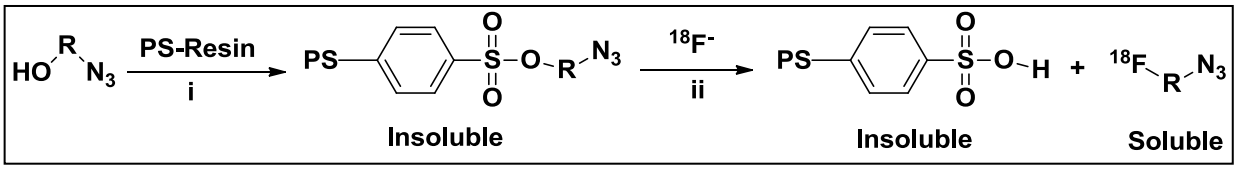


Figure 7. 3D MicroPET/CT images at 2 h post injection of male nude mice bearing CWR22Rv1 and PC3 tumor xenografts respectively **A)** [^{18}F]TG97 **B)** [^{18}F]AH-TG97 **C)** [^{18}F]AH2-TG97 Arrows indicates tumor placement.



Scheme 3. Azido-alcohol precursors preperation i) BH_3.THF, THF; 0 °C to RT; 15 h ii) NaN_3, DMSO; 15 h iii) $LiAlH_4$, THF; 0 °C to RT; 15 h iv) $SOCl_2$, DIPEA, DCM; 1h. Silica flash column purification and ^{1}H-NMR characterization were performed for each precursor.



Scheme 4. Synthetic sequence for [^{18}F]fluoro-azide preparation. i) 1:1 dichloromethane:pyridine; RT overnight ii) optimal labeling conditions were: DMF;150 °C; 30 mins.

Precursor	Heating Method*	K_2CO_3:K_{222} (mg)	RCY
1	Microwave	1:6.33	0%
2	Microwave	1 : 6.33 2 : 12.66 10 : 64	10-25% 30-40% 31%
2	Oil Bath	1 : 6.33 2 : 12.66	16-20% 26%
3	Microwave	1 : 6.33 2 : 12.66 10 : 64	7-15% 18% 34%
3	Oil Bath	1 : 6.33 2 : 12.66	16-25% 16%

Table 2. Investigation of different [^{18}F]fluoronation conditions of alcohol-azido precursors.
* All reactions were done in DMF at 150 °C for 30 mins

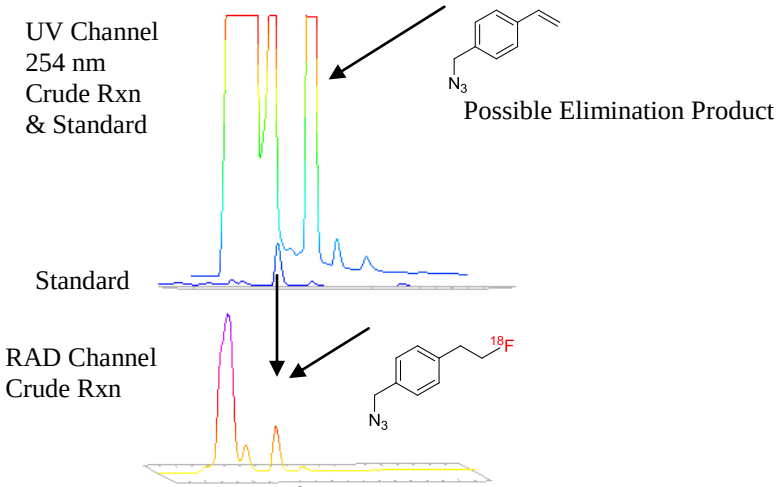


Figure 7. UV/RAD HPLC Precursor 2 ^{18}F Crude Reaction with overlay with the non-radioactive standard to product confirmation

Bibliography of Publications and Meeting Abstracts:

Personnel receiving pay from the research effort:

Shorouk F. Dannoon, Ph.D.

Manuscripts:

Writing is in progress for two manuscripts.

Meeting Abstracts:

1. **243rd ACS National Meeting Abstract:**

Potential One Step Labeling $^{18}F^-$ Prosthetic Group

Shorouk F. Dannoon[1], David Pham[1], Joseph E. Blecha[1], Cindy T. Lau[1], Michael Pun[1], Henry F. VanBrocklin[1]
[1]Department of Radiology and Biomedical Imaging, University of California San Francisco

Labeling peptides, proteins and antibodies with fluorine-18 ($^{18}F^-$) has been performed using prosthetic groups such as N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and N-[6-(4-[^{18}F]fluorobenzylidene)aminooxyhexyl]maleimide ([^{18}F]FBAM). However, these labeling methods often require multiple steps and results in low radiochemical yields. Consequently, the search has been ongoing to develop a one-step labeling prosthetic group. A new class of chloropyrimidine compounds with different electron withdrawing/donating groups at the position meta to the chloro leaving group were investigated. The $^{18}F^-$ radiolabeling was performed with the standard resolubilization method (drying the $^{18}F^-$ prior to labeling) and with the aqueous labeling conditions developed in our laboratory using the microfluidics. Labeling was achieved and the radiochemical yields varied as a function of the functional group and labeling method. The resultant [^{18}F]fluoropyrimdines were stable up to 24 hrs. This represents a promising single step labeling approach for new $^{18}F^-$ prosthetic groups.

2. **The 20th International Symposium on Radiopharmaceutical Sciences (ISRS)**

Radiofluorination of polymer-supported sulfonyl esters: Efficient preparation of labelled compounds

Dannoon, Shorouk [1]; Drake, Chris[1]; Wu, Lisa[2]; Berkman, Clifford E.[2], Jones, Ella F.[1], VanBrocklin, Henry F.[1]

[1]Department of Radiology and Biomedical Imaging, University of California San Francisco, USA.
[2] Department of Chemistry, Washington State University, USA.

Objectives: Techniques that minimize post radiolabeling purification offer the opportunity to increase the tracer effective specific activity and reduce synthesis time. Polymer-supported precursors provide a platform for tracer preparation by the nucleophilic displacement of the precursor from the resin by the radiohalogen. This leaves the insoluable polymer with the unreacted precursor that may be filtered from the solution leaving the tracer,

free of potentially reactive precursor, for subsequent reactions or in vitro/ in vivo use. This method has been employed previously to iodonate stannyl precursor (MIBG) supported on an insoluble polymer.[1] A method to prepare [^{18}F]fluoroazides, prosthetic groups for click chemistry, from polymer-supported sulfonyl esters has been explored. Radiofluorination followed by filtration or absorption and desorption onto a C18 Sep-Pak provides the desired reactive prosthetics.

Methods: Azido-alchohol precursors (Figure 1) were syntheszied from the corresponding bromo-alcohols and sodium azide (5-10 eq) at 90 °C overnight (Scheme 1). The purified (silica flash column) and characterized (NMR) azido-alcohols (5 eq) were coupled to a polystyrene sulfonyl chloride resin (1 eq) in 1:1 DCM:pyridine overnight at RT under N_2 gas. The coupling reaction was considered complete when a 1% bromophenol stain indicated no reactive chloride. The resin was washed and dried for storage. ^{18}F labeling of the resin bound azido-precursor was investigated under various conditions: solvents, heating method, temperature and reaction time. Labeling efficiency was determined by radioTLC and radioHPLC against non-radioactive standards.

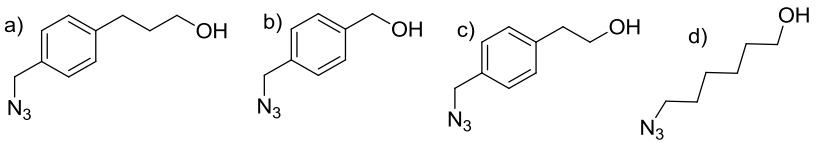
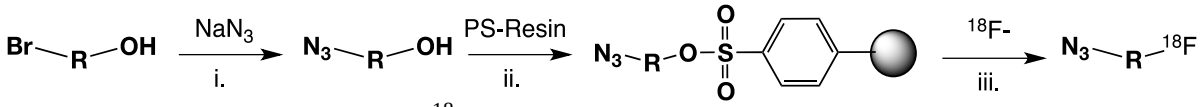


Figure 1. Azido precursors (a-d)



Scheme 1. Synthetic sequence for [^{18}F]fluoro-azide preparation. i.) DMF/90 °C overnight, ii) 1:1 DMC:pyridine/ RT overnight iii) DMF/150 °C for 30 mins.

Results: Azido precursors and their F-19 labeled standards were synthesized and characterized by ^{1}H-NMR. Complete coupling/loading on the resin was seen for all of the azido-alcohols. The labeling conditions of the investigated variables revealed DMF as solvent of choice and heating the reaction at 130 ºC for 30 min in a microwave was required. Nucleophilic $^{18}F^-$ labeling of the azido precursors on the resin was initiated by swelling the resin in DMF for at least 5 mins followed by the addition of the dried $^{18}F^-$, K_{222} and K_2CO_3 resolubilized in anhydrous DMF. The range of radiochemical yield for azides a, c and d (Figure 1) was 5-40%. Azide b (Figure 1) did not fluorinate. Fluorohexylazide was successfully coupled to an alkyne containing molecule

3. The 20th International Symposium on Radiopharmaceutical Sciences (ISRS)

"A high affinity 18F-labeled phosphoramidate peptidomimetic inhibitor as a PSMA-targeted PET imaging agent for prostate cancer"

Ganguly, Tanushree[1*]; Dannoon, Shorouk[2*]; Geruntho, Jonathan G.[1]; Hopkins, Mark R.[1]; Murphy, Stephanie Murphy; Cahaya, Hendry[2]; Blecha Joseph E[2].; Jivan, Salma[2]; Barinka Cyril[3]; Jones, Ella F.[2]; VanBrocklin, Henry F.[2]; Berkman, Clifford E.[1]

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Objectives: PSMA (prostate specific membrane antigen) is an enzyme biomarker that is highly up-regulated and expressed in prostate cancer cells, serving as an ideal target for imaging and therapeutic applications in prostate cancer. Previously our lead compound CTT-54 (IC_{50} = 14 nM), an *irreversible* phosphoramidate inhibitor of PSMA was labeled with ^{18}F and demonstrated *in vivo* PET imaging of prostate tumors in mice models [1]. For the current study, TG97 (a homolog of CTT-54, IC_{50} = 27 nM) was appended with a lipophilic linker (AH=aminohexanoic acid) and prepared for labeling with 18-flourobenzamide (^{18}FB) for PET imaging.

The ^{19}F- analog was co-crystallized with PSMA to obtain an insight on the effect of the protein-inhibitor binding interactions on overall uptake and bio-distribution of the compound.

Methods: TG97 was synthesized similar to previous compound with the exception of replacement of the P1 Serine of CTT-54 with 3-hydroxypropylglycine in TG97 [2] (Figure 1). Boc-Glu-OH was installed as the P2 residue which was removed under dry TFA to install the CBZ-AH-OH linker. After column purification, product was subjected to global hydrogenolysis under H_2/Pd conditions. Both ^{18}FB and ^{19}FB were installed as respective hydroxysuccinimidyl ester derivatives to yield the respective radiolabeled and cold compound. *In vitro* cell data (uptake and internalization) was obtained on LNCaP and 22RV1 (positive controls) and PC3 (negative control) cell lines. PET imaging and biodistribution studies were performed using nude mice xenografted with 22RV1 and PC3 tumors.

Results: ^{19}FB-AH-TG97 displayed high affinity for PSMA (0.4 nM, *irreversible*). Crystal structures data of PSMA co-crystallized with ^{19}FB-AH-TG97 confirmed an interesting interaction of the p-fluorophenyl moiety with a *remote arene-binding site* on the protein's surface [3]. *In vitro* cell and i*n vivo* bio-distribution data was also obtained for this compound. 22RV1 cell lines are known to express 10-fold less PSMA than LNCaP cells, which is consistent with our comparative *in vitro* cell uptake (2.8% in 22RV1 vs. 7.8% in LNCaP, 1h) and western blot data for both cell lines. Tumor uptake was observed with **no washout** up to 4h (Table 1), while rapidly clearing from blood and non-target organs with the exception on the kidneys (high PSMA expression is found in mouse kidneys).

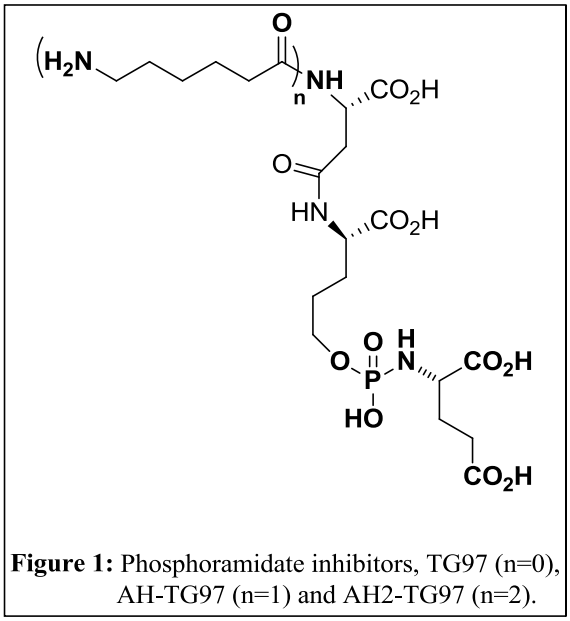

Figure 1: Phosphoramidate inhibitors, TG97 (n=0), AH-TG97 (n=1) and AH2-TG97 (n=2).

Compound	Tumor,1h	Tumor:Blood,1h	Tumor,2h	Tumor:Blood,2h	Tumor,4h	Tumor:Blood,4h
FB-CTT54 (LNCaP tumor)	-	-	1.24	9:1	-	-
FB-AH-TG97 (22RV1 tumor)	2.35	21.9:1	TBD	TBD	2.33	265.3:1

Table 1. Comparison of previous analog, FB-CTT54 with FB-AH-TG97

Conclusions: We successfully synthesized, labeled, and evaluated a new PSMA-targeted PET agent, FB-AH-TG97. The crystal structure of the compound with PSMA highlights its binding interactions which enables the rationalization of its prominent uptake as compared to our previous analog. The high uptake and exceptional clearance exhibited by this compound in the low PSMA-expressing cell line, 22RV1 now positions this PET imaging agent for clinical translation for prostate cancer and angiogenesis imaging.

Acknowledgements: Supported by National Institutes of Health (5R01CA140617 and T32GM008336).

References: [1]Lapi, S.E., et al., (2009) J Nucl Med. 50(12): p. 2042-8.[2]Nedrow-Byers, J.R., et al., (2011) The Prostate,72(8): p. 904-912.[3]Zhang, A.X., et al., (2010) J Am Chem Soc, 132(36): p. 12711-6.

4. 60th 2013 SNMMI Annual Meeting:

Fluorine-18 labeled PSMA-targeted phosphoramidate inhibitors as potential PET imaging agents for prostate cancer

Dannoon, Shorouk F.[1*]; Ganguly, Tanushree[2*]; Wu, Lisa[2]; Murphy, Stephanie[1]; Cahaya, Hendry[1]; Blecha Joseph[1]; Jivan, Salma[1]; Jones, Ella F.[1]; Berkman, Clifford E.[2]; VanBrocklin, Henry F.[1]

[1]Department of Radiology and Biomedical Imaging, University of California San Francisco, USA [2]Department of Chemistry, Washington State University, USA

*Both authors have contributed equally to this work.

Objectives : Prostate-specific membrane antigen (PSMA), a transmembrane protein commonly found on the tumor cell surface of late-stage, androgen-independent and metastatic prostate cancer, is an ideal biomarker for staging and targeted therapy. Previously reported FB-LW-54 (IC50 = 14 nM) is an 18F labelled irreversible phosphoramidate PSMA inhibitor, which demonstrated promising in vivo results in LNCaP tumor bearing mice [1]. Three more stable phosphoramidate-based PSMA inhibitor analogues with similar binding affinity as FB-LW-54 (TG97, AH-TG97 and AH2-TG97) were synthesized for radiolabeling with 18F-flourobenzamide (18FB) for PET imaging applications.

Methods: Succinimidyl [18F]fluorobenzoate (SFB) prosthetic group was prepared successfully either manually in lab or the Neptis box. SFB conjugation to the three analogues was carried out at pH 9.5, 37 °C for 10 min, purified by reversed phase HPLC and concentrated for in vivo studies. PET imaging (1 and 2 h) and biodistribution studies (1, 2 h and blocked with 250 ug LW-54) were performed using nude mice with 22RV1 (PSMA+) and PC3 (PSMA-) xenograft.

Results: SFB conjugation to the PSMA inhibitor analogues was accomplished in 27-45% radiochemical yields. Tumor uptake ranged between 1.7-2.4 %ID/g and can retain up to 2h. Minimal uptake in the non-target organs and rapid clearance from blood was noted. High uptake was observed in the mice kidneys as expected due to its normal expression. But PSMA expression in human kidney has not been identified. PSMA specificity was demonstrated in the tumor and kidneys by LW-54 blocking (56-93% uptake decrease).

Conclusions: We have successfully radiolabeled three PSMA-targeted analogues for mice xenografts ([18F]FB-TG97, [18F]FB-AH-TG97 and [18F]FB-AH2-TG97) and demonstrated their specificity. [18F]FB-AH-TG97 exhibited the highest tumor uptake with exceptional clearance in 22RV1 tumor bearing mice.

Research Support: Supported by DOD W81XWH-11-1-0464 (SFD) and W81XWH-11-1-0191 (LW) NIH 5R01CA140617 (CB).

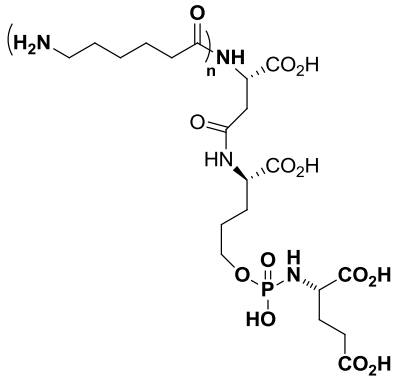

Figure 1: Phosphoramidate inhibitors, TG97 (n=0), AH-TG97 (n=1) and AH2-TG97 (n=2).

5. 2013 UCSF Annual Prostate Cancer Program Retreat

Fluorine-18 labeled PSMA-targeted phosphoramidate inhibitors as potential PET imaging agents for prostate cancer

Dannoon, Shorouk F.[1]; Ganguly, Tanushree[2]; Cahaya, Hendry[1]; Regan, Melanie[1]; Blecha Joseph[1]; Jivan, Salma[1]; Jones, Ella F.[1]; Berkman, Clifford E.[2]; VanBrocklin, Henry F.[1]

[1]*Department of Radiology and Biomedical Imaging, University of California San Francisco, USA*
[2]*Department of Chemistry, Washington State University, USA*

Objectives : Prostate-specific membrane antigen (PSMA), a transmembrane protein commonly found on the tumor cell surface of late-stage, androgen-independent and metastatic prostate cancer, is an ideal biomarker for staging and targeted therapy. Previously reported FB-LW-54 (IC50 = 14 nM) is an ^{18}F labeled irreversible phosphoramidate PSMA inhibitor, which demonstrated promising in vivo distribution and imaging results in LNCaP tumor bearing mice. Three phosphoramidate-based PSMA inhibitor analogues with similar binding affinity as FB-LW-54 (TG97, AH-TG97 and AH2-TG97) and enhanced stability were synthesized for radiolabeling with ^{18}F-flourobenzamide (^{18}FB) as improved agents for PET imaging.

Methods : Succinimidyl [^{18}F]fluorobenzoate ([^{18}F]SFB) prosthetic group was prepared in the Neptis box. SFB conjugation to the three analogues was carried out at pH 9.5, 40 °C for 15 min, purified by reversed phase HPLC and concentrated for in vitro and in vivo studies. In vitro cell uptake in CWR22Rv1 (PSMA+) and PC3 (PSMA-) and internalization in CWR22Rv1 (PSMA+) cells were done at 1 and 2 h post incubation at 37 °C and 5% CO_2. MicroPET/CT imaging at 2 h and biodistribution studies at 1 and 2 post injection were performed using nude mice with CWR22Rv1 (PSMA+) and PC3 (PSMA-) xenograft.

Results : SFB conjugation to the PSMA inhibitor analogues was accomplished in 50-60% radiochemical yields. Tumor uptake ranged between 1.6-3.2 %ID/g and can retain up to 2h. Minimal uptake in the non-target organs and rapid clearance from blood was noted. High uptake was observed in the mice kidneys as expected due to high PSMA expression. But PSMA expression in human kidney has not been identified.

Conclusions : We have successfully radiolabeled three PSMA-targeted analogues for mice xenografts ([^{18}F]FB-TG97, [^{18}F]FB-AH-TG97 and [^{18}F]FB-AH2-TG97). With their exceptional binding, tumor uptake and retention, and remarkable clearance from non-target tissues, these compounds are promising favorable candidates to move forward for human imaging.

Research Support: Supported by DOD W81XWH-11-1-0464 (SFD) and NIH 5R01CA140617 (CB/HFV).

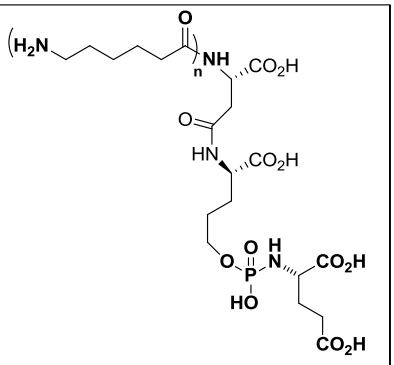

Figure 1: Phosphoramidate inhibitors, TG97 (n=0), AH-TG97 (n=1) and AH2-TG97 (n=2).

Figure 2: 3D microPET/CT overlay of CWR22Rv1 tumor bearing male nude mice 4 h post injection of 200 uCi [^{18}F]FB-AH-TG97